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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

COX et al.

Application No.: 09/229,037

Filed: January 12, 1999

Title: REGULATION OF ENDOGENOUS GENE EXPRESSION IN CELLS USING ZINC FINGER PROTEINS

Examiner: J. Brusca

Group Art Unit: 1631

DECLARATION UNDER 37 C.F.R. § 1.132

I, Andreas Reik, declare as follows:

1. I am presently a Scientist and Team Leader of the Gene Activation Group at Sangamo BioSciences, Inc. and have been at Sangamo since August, 2000. From 1994-2000, I was a postdoctoral fellow in the laboratory of Dr. Mark Groudine at the Fred Hutchinson Cancer Research Center, Seattle, WA where I studied the role of the Locus Control Region in the regulation of the chromatin structure and expression of the human β–globin locus. In 1993, I received a Ph.D. Degree in Biology from the University of Heidelberg, Germany. My thesis research concerned analysis of the molecular mechanisms governing hormone induction of expression of the tyrosine aminotransferase gene, and was directed by Professor Günther Schütz.

2. My current work at Sangamo involves the use of engineered zinc finger proteins (ZFPs) for activation of gene expression. In the course of this work, my co-workers and I generated several constructs encoding fusion proteins comprising an engineered ZFP and two or more regulatory domains. In some cases, these fusion proteins comprise multiple copies of the same regulatory domain; in others, they comprise different regulatory domains.

3. The data presented herein show that fusion proteins comprising an engineered zinc finger protein and two or more activation domains activate gene expression.

Furthermore, the data show that gene expression is activated when the zinc finger protein is associated with two or more of the same domains or with different activation domains.

4. A zinc finger binding protein, designated "ZFP1" was engineered to recognize a target site in the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene. The K_d for the ZFP1 zinc finger protein is 0.05 nM. Separate fusion constructs were made, in which the ZFP1 binding domain was fused to a VP16 activation domain, a p65 activation domain, three copies of the p65 activation domain, or one copy of a VP16 activation domain and one copy of a p65 activation domain. Each construct also contains sequences encoding a nuclear localization signal and an epitope tag. Each construct was transfected into A549 lung cancer cells, and GM-CSF mRNA levels were measured and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The results, shown in Figure 1, indicate that fusion proteins comprising two or more activation domains function in cells to activate GM-CSF gene expression.

5. A zinc finger binding protein, designated "ZFPA" was engineered to recognize a target site in the cannabinoid receptor-1 (CNR1) gene. The K_d for the ZFPA zinc finger protein is 0.06 nM. Separate fusion constructs were made, in which a VP16 activation domain was fused to the C-terminus of the ZFPA binding domain, a VP16 activation domain was fused to the N-terminus of the ZFPA binding domain and a p65 activation domain was fused to the C-terminus of the ZFPA binding domain, or a p65 activation domain was fused to the N-terminus of the ZFPA binding domain and a VP16 activation domain was fused to the C-terminus of the ZFPA binding domain. Each construct also contained sequences encoding a nuclear localization signal and an epitope tag. Each construct was transfected into HEK293 (human embryonic kidney) cells, and CNR1 mRNA levels were measured and normalized to 18S rRNA. The results, shown in Figure 2, indicate that fusion proteins comprising two or more activation domains function in cells to activate CNR1 gene expression.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of

the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 9/4/02 Signature: _____

Andreas Reik, Ph.D.

FIGURE 1

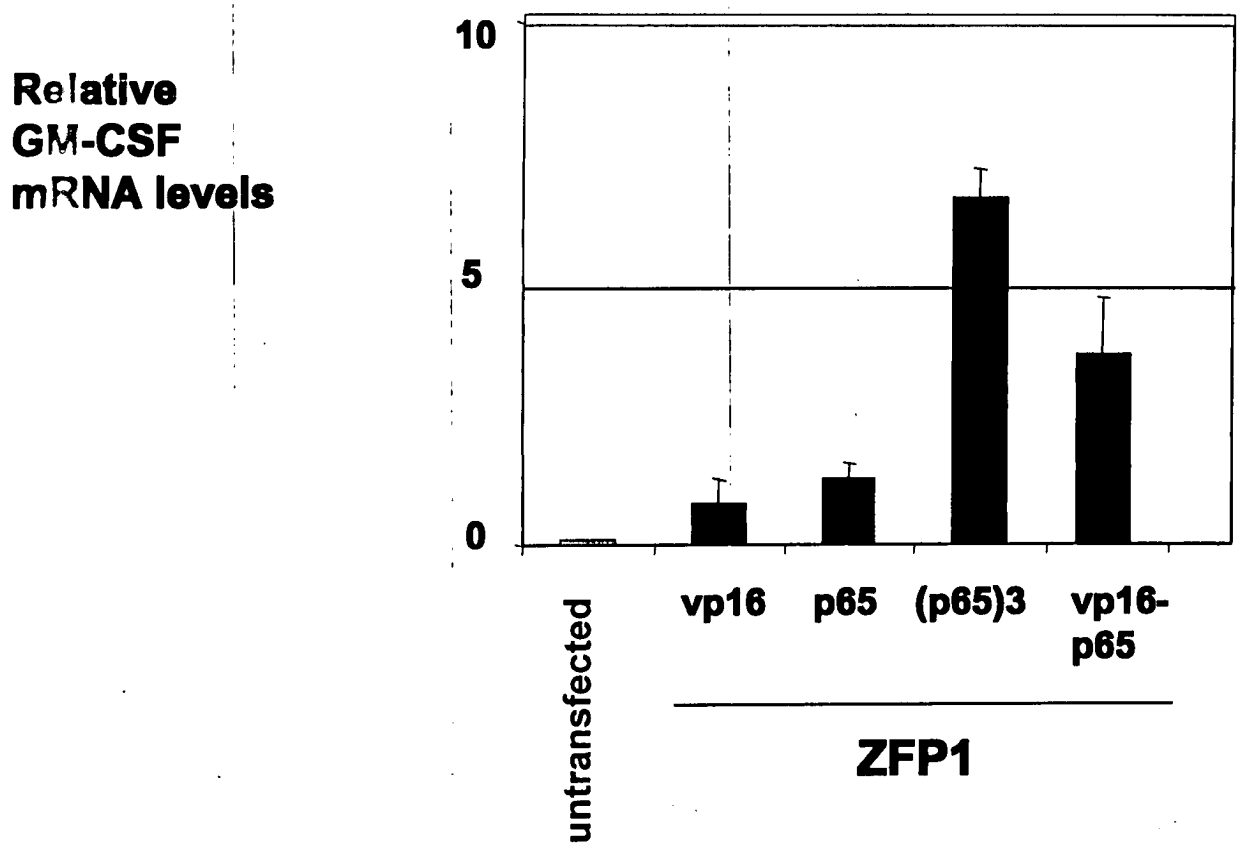
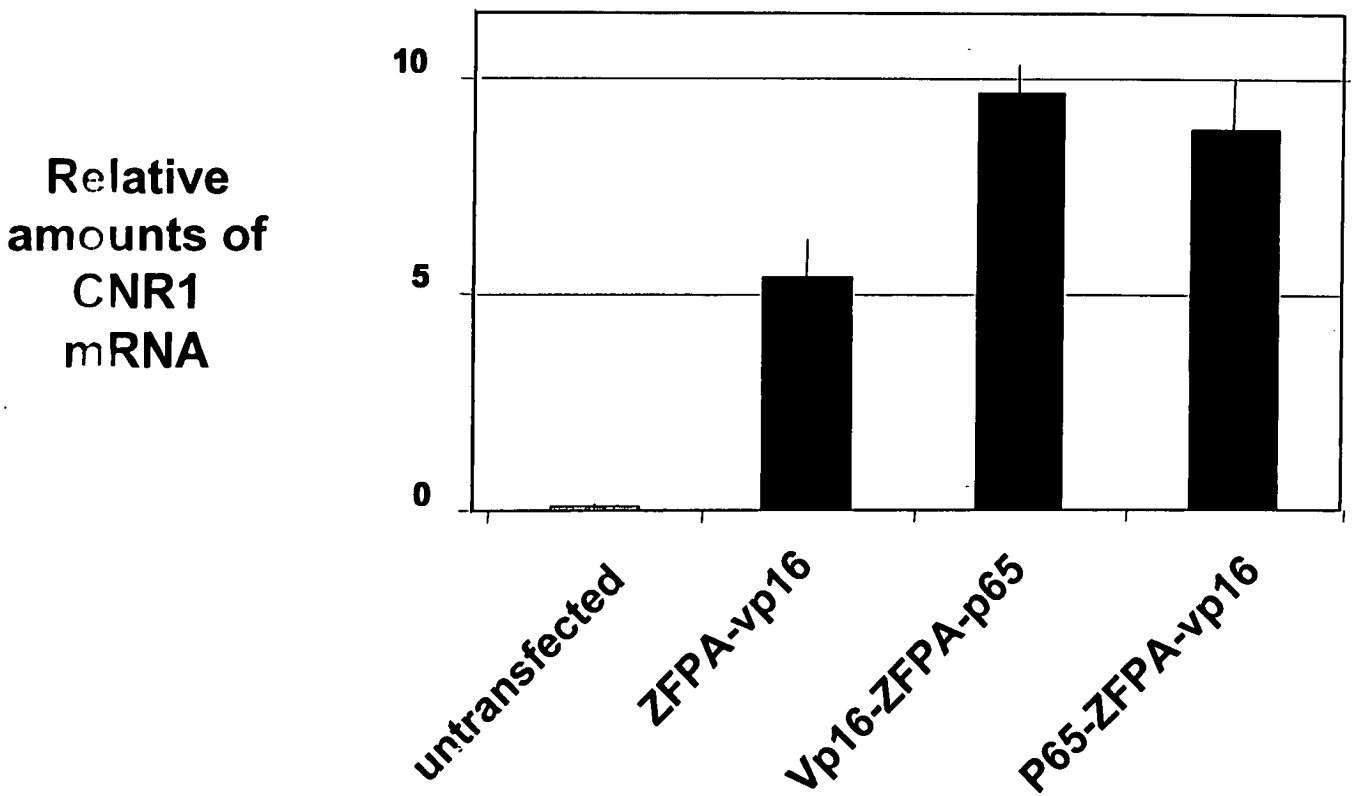


FIGURE 2



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